PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

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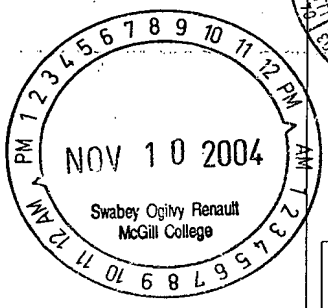

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	02.11.2004
Applicant's or agent's file reference 14149-11PCT MG	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA 03/01141	28.07.2003	29.07.2002

Applicant
UNIVERSITE LAVAL et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Faux, K Tel. +49 89 2399-8062

Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
14149-11PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA 03/01141	28.07.2003	29.07.2002
International Patent Classification (IPC) or both national classification and IPC		
C12N15/82		
Applicant		
UNIVERSITE LAVAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.02.2004	02.11.2004
Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Bilang, J Telephone No. +49 89 2399-8707

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA 03/01141

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-27 as originally filed

Claims, Numbers

1-24 received on 31.08.2004 with letter of 26.08.2004

Drawings, Sheets

1/9-9/9 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-21
	No:	Claims	1-3,22-24
Inventive step (IS)	Yes:	Claims	7-14, 17, 19-21
	No:	Claims	1-6,15,16,18, 22-24
Industrial applicability (IA)	Yes:	Claims	1-24
	No:	Claims	

2. Citations and explanations

   see separate sheet

1. The present application discloses a method for increasing the recovery of recombinant proteins by expression of plant protease inhibitors in plants.

2. D1 (WO99/36516) discloses the induction of endogenous proteinase inhibitors for decreasing the degradation of heterologous proteins (p 10, l 6-17). There is no doubt that the inhibitor as well as the proteinase is released from the plant cell upon disruption of said cell. D1 thus anticipates the subject-matter of at least claims 1-3 (Article 33(2) PCT)

   D2 (WO99/38987) discloses the production of antitrypsin in plant cells. These cells cannot be distinguished from the cells claimed in claims 22-24 (Article 33(2) PCT).

   The arguments of the applicants as submitted with the letter dated 26.08.2004 have been taken into account, but were not deemed to be convincing in the light of the broad formulation of the claims. Claim 1, for example, refers to significant alterations of the natural physiology. It would appear that a plant can be treated with jasmonate to induce natural protease inhibitors without "significantly" altering the natural physiology.
   Similarly, claim 22 is silent about the nature of the protease or the recombinant protein.

3. The skilled person knows that proteinases which are released upon disruption of plant cells degrade (recombinant) proteins. It does not require inventive activity to recognize that the teachings of D1 are applicable to such proteases as well, as long as endogenous inhibitors of these proteases are known. The broad claims 1-6, 15, 16, and 18 therefore are not considered to be based on an inventive activity (Article 33(3) PCT).

CLAIMS

1. A method for increasing the recovery yield of a recombinant protein in plant cells without significantly altering the natural physiology of said plant cells, comprising neutralizing the activity or the action of at least one plant protease involved in the degradation of said recombinant protein with an inhibitor released from said plant cell at the time said plant cells are disrupted.

2. The method of claim 1, wherein said plant cells are from a plant or from an *in vitro* culture.

3. The method of claim 1 wherein said neutralizing is partial or total.

4. The method of claim 1 wherein said neutralizing occurs when processing said plant cells for extracting said recombinant protein.

5. The method of claim 1, wherein said plant cells are disrupted when performing a process for extracting said recombinant protein.

6. The method of claim 1, wherein said protease is selected from the group consisting of a cysteine protease, an aspartate protease, a metallo protease, a serine protease, a threonine protease, and a multispecific protease.

7. The method of claim 1, wherein said inhibitor is recombinantly produced in said plant cells transformed with an expression cassette comprising a promoter operably linked thereto.

8. The method of claim 1, wherein said inhibitor is linked to a leader peptide, a signal peptide or an anchorage peptide or a protein to lead or anchor said inhibitor to a cell part or extracellular compartment in a manner to protect said

recombinant protein from the activity of a plant protease during the extraction process.

9. The method of claim 7, wherein said inhibitor does not interfere with the activity of said protease to preserve the physiology or the growth of said plant cells or plant containing said plant cells.

10. The method of claim 7, wherein said cell part is an organelle selected from the group consisting of a mitochondria, a chloroplast, a storage vacuole, the endoplasmic reticulum, and the cytosol.

11. The method of claim 7, wherein said inhibitor is selected from the group consisting of an antibody or a fragment thereof, a sens-mRNA or anti-sens mRNA, an inhibitor of transcription or a regulator thereof, an inhibitor of translation or a regulator thereof, an inhibitor of leading or signal peptide, an inhibitor of metabolic acquisition of activity of a protease, a protease-specific protease, and an affinity peptide protease leading to segregation to said protease into an organelle or a cell compartment.

12. The method of claim 8, wherein said genetically altered plant is an alfalfa or a potatoe.

13. The method of claim 1, wherein said protease is chymostatin-sensitive serine protease.

14. The method of claim 1, wherein said protease is a cystatin-sensitive cysteine protease.

15. The method of claim 1, wherein said inhibitor is a protease inhibitor.

16. The method of claim 1, wherein said plant cells are genetically altered

17. The method of claim 1, wherein said neutralizing is performed by an inhibitor encoded by a gene under control of a constitutive or an inducible promoter or a tissue or development specific promoter.

18. The method of claim 3 or claim 5, wherein said recombinant protein or inhibitor are produced in nucleus or plastids of said plant cells.

19. A method for increasing the recovery yield of a recombinant protein in a plant comprising the steps of:

a) allowing production of a recombinant protein in plant cells genetically altered for modulating at least one genetic or metabolic reaction to partially or totally neutralize action or activity of at least one protease at the time of disrupting of said plant cells; and

b) recovering said recombinant protein after disrupting of said plant cells.

20. The method of claim 19, wherein said plant cells are from a plant or from *in vitro* culture.

21. The method of claim 19, wherein said action or activity of said protease is neutralized by inhibiting its transcription or translation into an active protease, or by an inhibitor produced by said plant cells, or linking said recombinant protein with a peptide or protein in manner to protect said recombinant protein from the action or activity of said protease.

22. A plant cell or a plant genetically altered to modulate at least one genetic or metabolic reaction to partially or totally neutralize the action or activity of at

least one protease for improving the recovery of a recombinant protein from said plant cell or plant at the time said plant cell or cells of said plant are disrupted.

23. The plant cell or plant of claim 22, wherein said modulation inhibits the transcription or translation of a gene encoding for a protease, or neutralizes a protease with a protease inhibitor produced in said plant or plant cell.

24. The plant cell or plant of claim 22, wherein said recombinant protein or protease inhibitor is linked to a leader peptide, a signal peptide or protein in manner to improve protection of said recombinant protein from at least one protease.